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13. ABSTRACT ***(Maximum 200 Words)***

We attempted to identify phage display cDNA clones that exhibited proteins with carbohydrate affinity, using libraries constructed in mid copy display T7Select 10-3 vector. Although we hoped that higher affinity with multiple number of capsid fusions would facilitate the screening of those clones, the attempts have been unsuccessful.

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Introduction:

In multicellular organisms, each cell is surrounded by other cells as well as a complex network of extracellular matrix. On the outer membrane, complex carbohydrate structures are present as parts of glycoproteins and glycolipids. These carbohydrate structures play an important role in interacting with proteins (lectins) (1) and other carbohydrate structures (2).

In an attempt to identify and clone unknown human proteins with carbohydrate affinity, we proposed to employ the novel technology named phage display (3). The phage display technology is based on the surface expression of the peptide sequences fused with phage capsid protein, and has been most successfully used in cloning phage particles that express variable domains of antibodies specific to certain antigens (4,5). Phage display peptide libraries made with synthetic oligonucleotides have also been utilized to identify peptide sequences that interact with a variety of bait ligands, such as proteins, peptides, DNAs, RNAs, and oligonucleotides (6,7). We have used the T7 phage cDNA display system developed by Novagen (Madison, WI) (8). Different from filamentous phage systems where the peptide sequences are fused with capsid proteins at the C-terminus, the T7 system allows the fusion of protein sequences up to 1200 amino acid residues long fused with gene 10 capsid protein at the N-terminus of proteins.

We constructed a phage display cDNA library using RNA from cells that stably expressed A transferase, and performed biopanning experiments using, as a bait ligand, crude mucin fraction containing blood group H-specific glycoproteins. Although no enrichment of the phages that expressed A transferase fusion protein was observed, selective augmentation was observed of the phages that expressed the fusion proteins with galectin-3, a soluble β-galactoside-binding (S-type) lectin (9). Because of this lectin's known affinity with the blood group-specific oligosaccharides (10,11), the results demonstrated that the phage display was useful in cloning cDNAs encoding a protein with binding capacity to carbohydrates.

Body:

During the originally proposed two years, we tried, without success, to identify cDNA clones that encode unknown proteins with carbohydrate affinity using the T7 phage system. The cDNA display libraries constructed in the T7Select 1-1 vector were primarily used in the screenings. Since the T7Select 1-1 vector displays a low copy number (0.1-1 capsid fusion per phage) of peptides or larger proteins, there was a possibility that the affinity was too weak with this system for the detection of ynknown carbohydrate-binding protein(s). Because the new vector, T7Select 10-3 vector, was developed for mid copy number display (5-15 capsid fusions per phage) of peptides and proteins, and has become available, we have repeated some of the screening experiments using the libraries constructed in this mid copy vector in the no-cost extension period. Although we hoped to identify candidate novel lectin(s) using this T7Select 10-3 vector with higher affinity, no promising candidates have been obtained.

Key Research Accomplishments:

None

Reportable Outcomes:

None

Conclusions:

Although we re-tried to identify phage clones that express fusion proteins with affinity to carbohydrate ligands using mid copy display vector, the attempts have been unsuccessful. The reason for our failure is unclear. However, different from galectins, the lectins of other family may lose the carbohydrate binding affinity by the fusion at the N-terminus. It is possible that the expression of those fusion proteins may be toxic to bacteria and the phages that express those proteins may be eliminated from the population in the library. It is also possible that those proteins are instable and may not be displayed on phage particles.

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